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Efficacy of *Metarhizium Anisopliae* and *Bacillus Thuringiensis* against Tomato Leafminer *Tuta Absoluta* Meyrick (Lepidoptera : Gelechiidae)

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Abstract

Susceptibility of *Tuta absoluta* Meyrick (Lepidoptera : Gelechiidae) populations to *Metarhizium anisopliae* and *Bacillus thuringiensis* (Bt) were evaluated under laboratory conditions. *T. absoluta* larvae were treated either individually or in combination with a single dose rate of *B. thuringiensis* (0.5 µL / L) and three conidial suspensions *viz*. 1 x 10^4, 1 x 10^6 and 1 x 10^8 spores / mL of *M. anisopliae*. Larval mortality, pupation, adult emergence, mycosis and sporulation varied depending on the application of different quantities of *M. anisopliae* alone and in combination with *B. thuringiensis*. Maximum mortality (100%) was achieved in 2nd instar larvae when *M. anisopliae* (1 x 10^8 spores/mL) and *B. thuringiensis* (0.5µL / L) were applied synergistically, while 4th instar larvae recorded a 95.45% mortality. Compared with the untreated checks, mortality, pupation and adult emergence of both 2nd and 4th instar larvae were significantly reduced with the combined application of *M. anisopliae* (1 x 10^8 spores / mL) and *B. thuringiensis* (0.5µL / L). Mycosis was most prevalent on 2nd and 4th instar larvae, exceeding 88 and 80% respectively, after exposure to 1 × 10^4 conidia / mL. The results indicate that the entomopathogenic fungi and the insecticidal protein produced by *B. thuringiensis* can be used in combination as biocontrol agents for the management of *T. absoluta*.

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular and nutritious vegetable crops in Greece. Application of synthetic insecticides has increased and remains the most common pest control strategy. However, chemical control is difficult due to the rapid development of pest resistance to insecticides as well as due to the negative impact on natural enemies, the environment and human health. Considering all the above factors, the scientific community has increased its interest in alternative control methods and Integrated Pest Management approaches.[1-3]

Among the possible methods for the management of *Tuta absoluta*,[4-7] which is a key pest of tomato, the use of entomopathogenic microorganisms provides an alternative to chemical insecticides with increased environmental safety and pest selectivity; hence they can be used either alone or in combination with other pest control tactics. Moreover, microbial agents facilitate the survival of beneficial fauna due to their high target specificity. The entomopathogenic fungus *Metarhizium anisopliae* and the bacterium *Bacillus thuringiensis* have an important role in crop protection, and may represent effective and ecologically sound solutions to pest problems.[9,10]

M. anisopliae mainly uses propagules such as conidia, blastospores or hyphae to infect the host by direct contact; however, secondary infections took place by horizontal transmission of spores from mycosed cadavers.[12] Unlike other microbial agents which need to be ingested to manifest their action, the mycospores adhere to and infiltrate the cuticle upon contact, thus growing internally and producing different toxins that kill the insect pests.[10,13] *B. thuringiensis* is an endospore forming bacterium that produces parasporal crystalline proteinaceous inclusions (Cry and Cyt toxins) during its sporulation and stationary growth phases.[14,15] The primary effect of Cry toxins is the cessation of feeding due to paralysis of the mouthparts and gut, leading to the formation of pores in the apical microvilli membrane of the target cells, which results in the lysis of midgut epithelial cells and septicemia.[15,16] The specific and narrow spectrum of action of these toxic crystal proteins against lepidopteran, dipteran and coleopteran insects[17,18] renders their use in food and other sensitive crops safer than chemicals which may cause severe hazardous effects.[9,19,20] Several studies revealed that in the case of lepidopterous pests, commercially available *B. thuringiensis* enhanced the efficacy of entomopathogenic fungi when used in an integrated fashion.[21,22] The enhanced synergistic effect between the two agents could be due to larval starvation, as it is likely that the bacteria arrest the nutrition of insects, thus enabling the fungus to kill the weakened worms more quickly.[23]

Keeping in view the significance of these promising alternatives, the current experiment was planned to evaluate the single and combined effects of *M. anisopliae* and *B. thuringiensis* on mortality, pupation and adult emergence of *T. absoluta*.

Materials and Methods

Six-week-old tomato plants were used in the experiments. The plants were grown in a climatic chamber (24 ± 1 °C, R.H. 65%, photoperiod 16L: 8D; a nutrient solution was applied daily). *T. absoluta* was reared on tomato plants in growth chambers (24 ± 1 °C, R.H. 70%, photoperiod 16L : 8D), inside cages (55 × 75 × 80 cm). During the experiments, *T. absoluta* larvae were reared on tomato leaves at 22.8 - 24.0 °C, 12h photophase and R.H. 82 - 100%. *T. asboluta* larvae were initially collected from tomato fields in Mirtia, Ilia Greece (37.702267, 21.359392), for laboratory mass rearing.

M. anisopliae was collected from different regions in Western Greece and different hosts/sources. The specific strain was selected for its high entomopathogenicity, as resulting from preliminary assessment (unpublished data). In order to prepare the appropriate suspensions for the experiments, the isolates were grown in 9-cm Ø Petri dishes with Sabouraud Dextrose Agar and kept in the dark for 15 days at 25 °C ± 1. For bioassays, fresh conidia were collected from 15 day-cultures. Conidial suspensions were prepared by scraping the surface of the Petri dish using a sterile loop and transferring the conidia into a 500 mL glass beaker containing 50 mL sterile distilled water plus 0,05% Tergitol® NP9. The conidial suspension was panned across several layers of sterile cloth and mixed with a magnetic stirrer for 5 min.[3,24] Subsequently, a Neubauer hemocytometer was used to determine the conidial concentration under a phase contrast microscope at 400 x magnification.

For the bacterial treatments, we used Bactospeine®, a microbial insecticide from *Bacillus thuringiensis* sub *kurstaki* (Hellafram A.E, Greece), formulated as granules and wettable powder (WP) and with 32.000 IU / mg potency. Aqueous suspensions of each dose were prepared at the desired concentrations. The powder was mixed with water in a sterilized Erlenmeyer flask (100 mL), using a sterilized spatula. Then, aqueous suspensions were prepared by mixing the solution with a magnetic stirrer for 3 min.

Bioassay

The mortality of 2nd and 4th instar larvae, pupation and adult emergence was determined by treating *T. asboluta* larvae with a single dose rate of *B. thuringiensis* (0.5 µL / L) and three different concentrations of *M. anisopliae* (*Ma*1: 1 x 10^4, *Ma*2: 1 x 10^6, *Ma*3: 1 x 10^8 conidia / mL), both individually and in their respective combinations (Bt + *Ma*1, Bt + *Ma*2 and Bt + *Ma*3). *B. thuringiensis* was applied by dipping tomato leaf discs (3 cm each) from four-week old seedlings into the bacterial suspension at a dose rate of 0.5 µL / L, for 3 minutes, in a petri dish. Treated tomato leaves were offered to the larvae in a sterilized petri dish for 48 hours. Larvae were then offered the fresh untreated maize leaves until they pupated or died. Before the treatment with *B. thuringiensis*, tomato leaves were washed in a solution of commercial bleach (3% sodium hypochlorite) for 2-3 minutes to remove any kind of debris or disease-causing agent, as well as in dd water for one minute, and they were then allowed to dry. However, *M. anisopliae* was applied following the larval immersion method.[25] To check the synergistic effect of *M. anisopliae* and *B. thuringiensis*, larvae were exposed to *B. thuringiensis* by feeding them with treated tomato leaves for 48 hours, and they were then dipped in the fungal suspension for 10 seconds.[26] The larvae were then allowed to feed on fresh untreated tomato leaves until they pupated or died. Larval mortality, pupation rate, adult emergence, mycosis and sporulation were recorded. The experiment was carried out in a completely randomized design using 15 larvae of both 2nd and 4th instars per replicate, and the bioassay was repeated three times.

Mycosis and Sporulation

For mycosis and sporulation, dead *T. absoluta* individuals from each treatment were collected, and they were counted and refrigerated at 4 °C in plastic vials. Prior to three washings with distilled water, surface sterilization of mycosed cadavers was done with sodium hypochlorite solution (0.05%) for 2-3 min. The cadavers were placed on Sabouraud Dextrose Agar plates and incubated at 25 ± 2 °C and 75% RH for 7 days. The insects showing external fungal growth were determined under microscope. Sporulation data were determined by immersing mycosed cadavers from each replication in 20 mL distilled water with a drop of Tween-80.[27] The treatments were replicated independently, three

Table 1: Mean effect of *M. anisopliae* (Ma) and *Bacillus thuringiensis* (Bt), alone and in combination, on mortality, pupation and adult emergence of 2nd and 4th larval instars of *T. asboluta*. Means sharing the same lower-case letters are not significantly different from each other at the significance level

Treatments	Mortality (%)		Pupation (%)		Adult emergence (%)	
	2nd Instar	4th Instar	2nd Instar	4th Instar	2nd Instar	4th Instar
*Ma*1	21.08e	13.15f	75.00ab	79.16ab	65.38b	70.83b
*Ma*2	29.89de	25.05ef	65.27bc	72.21bc	43.06c	51.38c
*Ma*3	52.58cd	43.30cd	41.67de	54.17de	15.78e	29.14de
Bt	41.68cde	30.98de	54.17cd	63.88cd	26.39d	31.94d
*Ma*1 x Bt	65.23bc	56.43bc	31.94ef	40.27e	8.31ef	15.21ef
*Ma*2 x Bt	80.18ab	71.76b	18.05fg	23.61f	2.79f	6.96fg
*Ma*3 x Bt	100.00a	95.45a	0.00g	4.19g	0.00f	0.00g
Control	0.00g	0.00g	91.61a	93.03a	86.11a	87.50a

times. The solution was then thoroughly stirred, and the total number of conidia / mL was counted with the help of a haemocytometer, under microscope.

Statistical Analysis

Corrected percent mortality was calculated using Abbott's formula[28] and prior to analysis these values were arcsine transformed. The data regarding larval mortality, pupation, adult emergence, mycosis and sporulation were subjected to IBM (SPSS Inc., IL, USA, version 23.0.) (SAS Institute 2013) through one-way analysis of variance (ANOVA). The Bonferroni test was used for separating means at a 5 % significance level.[3]

Results

Larval Mortality

A high mortality trend of *T. asboluta* larvae was observed in the combined treatments of *M. anisopliae* and *B. thuringiensis*, and as the dose rate increased. A significant maximum mortality of 100% and 95.45 % was achieved in 2nd and 4th instar larvae respectively, when *M. anisopliae* (1 x 10^8 conidia / mL) and *B. thuringiensis* (0.5 µL / L) were used in combination (Table 1). Minimum mortality of 2nd (21.08 %) and 4th instar larvae (13.15%) was recorded in the *Ma*1 treatment (1 x 10^4 conidia of *M. anisopliae* per mL) and was significantly different from the combined applications of *M. anisopliae* and *B. thuringiensis*. Similarly, *B. thuringiensis* produced a significantly lower mortality of 2nd (41.68%) and 4th (30.98%) instars when compared with the combined application of both entomopathogenic agents. The maximum recorded mortality was followed by a mortality of 80.18% and 71.76% of 2nd and 4th instar larvae respectively, in *Ma*2 and *B. thuringiensis* combination treatments. No larval mortality was observed for any instars in control treatments. Moreover, 2nd instar larvae were found to be more death susceptible than 4th instar larvae, as mortality was found to be linearly related to their developmental stage in all tested treatments (Table 1).

Pupation and Adult Emergence

A significantly lower pupation and adult emergence was recorded in tested larval instars of *T. absoluta* in those treatments where the mortality rate was high (Table 1). In the control, 91.61% and 93.03% of 2nd and 4th instar larvae successfully transformed into pupae, followed by 75.00% and 79.16% pupation when 2nd and 4th instar larvae were treated with *M. anisopliae* at a concentration of 1 × 10^4 conidia/mL (*Ma*1). Conversely, minimum pupation was observed in the case of both 2nd (0%) and 4th (4.19%) instars when the highest concentrations of *M. anisopliae* (1 × 10^8 conidia/mL) and *B. thuringiensis* were applied simultaneously (*Ma*3 x Bt) (Table 1).

Similarly, besides the control, maximum adult emergence was observed in larvae of 2nd (65.38%) and 4th instars (70.83%) when treated with *M. anisopliae* (1 × 10^4 conidia / mL); however, no adult emergence was recorded in both 2nd and 4th instars when they were treated with a combination of the highest dose rate of *M. anisopliae* (1 × 10^8 conidia/ mL) and *B. thuringiensis* (0.75 µL / L) (Table 1).

Mycosis and Sporulation

Differences in mycosis were noted in the tested population of *T. absoluta* at various conidial concentrations. Maximum mycosis was observed in 2nd (88.89 %) and 4th (81.09 %) instar larvae when treated with the lowest concentration (1 × 10^4 conidia/ mL) of *M. anisopliae*, while it gradually decreased when both instars were treated with a higher dose of *M. anisopliae*, individually and in combination with *B. thuringiensis*. However, the combination of *B. thuringiensis* with *M. anisopliae* at a concentration of 1 × 108 conidia / mL (*Ma*3) demonstrated a minimum mycosis of 18.06% and 14.65% in 2nd and 4th instar larval cadavers respectively. Similarly, sporulation in cadavers of both larval instars was higher when low concentrations of *M. anisopliae* conidia were used. A significantly higher sporulation of 142.67 and 128.83 conidia/mL in 2nd and 4th instars cadavers was recorded in the *Ma*1 (1 × 10^4 conidia / mL) treatment. However, minimum sporulation of 87.67 (conidia / mL) in 2nd and 73.31 (conidia / mL) in 4th instar larvae was documented at the high dose of *M. anisopliae* in combination with *B. thuringiensis*.

Discussion

The aim of the present study was to assess the effect of *M. anisopliae* and *B. thuringiensis* against 2nd and 4th larval instars of *T. absoluta* both individually and synergistically. Both larval stages showed varied mortality responses to various concentrations of fungi, alone and in combination with *B. thuringiensis* as used in this study. Individual

applications of entomopathogenic fungi have a great potential to suppress lepidopterous pests.[29] This was corroborated by our findings in which *M. anisopliae* showed significant mortality efficiency (> 40%) at the highest dose rate, in both 2nd and 4th instar larvae, especially in the 2nd instar larvae whose mortality exceeded 50%. Mortality of older instars occurs via infection from mycosed cadavers inside the stem.[30] Similar effectiveness of *M. anisopliae* was recorded by Nguyen *et al*.[31] in their laboratory bioassays, against the various larval instars of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). In the current experiment, a dose-dependent upsurge in mortality is corroboratory to the study of Sasidharan and Varma,[32] who reported higher larval mortality of Indarbela quadrinotata Walker (Lepidoptera: Cossidae)-up to 100% - when treated with higher doses of *Beauveria bassiana* (Bals.-Criv.) Vuill., compared to 66.7 % of mortality when treated with lower fungal concentrations.

The declining trend regarding mortality from 2nd to 4th instar larvae was recorded for each treatment application in the current study. Similar outcomes were recorded by Inglis *et al*.,[33] who observed that the virulence of entomopathogenic fungi varies with the different developmental stages of insects. This can be attributed to the reduced fungal germ tube penetration inside the insect body because of the enhanced melanin contents in the mid gut and cuticle of the insect.[34] Similar findings were also presented by Hafez *et al*.,[35] where *B. bassiana* was more virulent to neonate larvae than older instars of the potato tuber moth *Phthorimaea operculella* (Z.) (Lepidoptera: Gelechiidae). Vandenberg *et al*.[36] on the other hand, found that the 2nd instar larvae of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) were more resistant to entomopathogenic fungi than the 3rd and 4th instars.

In the present findings, virulence of *B. thuringiensis* toxin was in inverse relation with the growth and development of *T. absoluta* larvae. Similar decrease in the efficacy of *B. thuringiensis* against *Helicoverpa zea* Boddie (Lepidoptera:Noctuidae) as the larvae grew up was reported by Herbert and Harper.[37] Similarly, after 96 hours of *B. thuringiensis* application, Zehnder and Gelernter[38] reported a mortality of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), ranging from 40 to 98% in 2nd instars compared with 52% in 3rd instars. Moreover, Lacey *et al*.[39] reported that the control of the Colorado potato beetle ranged from good to excellent with the application of low to high concentrations of *B. thuringiensis* respectively. Enzymatic activity is responsible for differences in mortality between different larval instars. It has been stated that the action of detoxification enzymes changes significantly within and among different developmental stages. This action is minor in the egg stage, increases with each nymphal or larval stage and reduces again to zero at the pupal stage.[40,41]

These results indicate clearly that a significantly higher larval mortality was observed in both 2nd and 4th instar larvae when *M. anisopliae* was synergized with *B. thuringiensis*. These outcomes are in accordance with the findings of Lacey *et al*.[39] who also reported that the highest larval mortality of the Colorado potato beetle occurred in the plots treated with the combined use of *B. thuringiensis* and entomopathogenic fungi, while the lowest mortality was recorded in untreated checks. Similarly, the combined application of *B. bassiana* and *B. thuringiensis* caused significant larval mortality of the Colorado potato beetle, which was not the case when they were individually applied.[42] Lewis *et al*.[43] concluded that the integrated use of *B. thuringiensis* and *B. bassiana* enhanced the larval mortality of the European corn borer, Ostrinia nubilalis Hübner (Lepidoptera: Crambidae). The synergistic action of entomopathogenic fungi and bacteria was further supported by Gao *et al*.[21] who reported that the starvation stress triggered by *B. thuringiensis* intoxication may cause negative effects on host immunity and physiology. The starvation stress also enhanced the inter-molt period, which could be the possible cause for the improved vulnerability of the *L. decemlineata* larvae.[44] Additionally, Lawo *et al*.[45] found that sub lethal Cry2Aa intoxication of *B. thuringiensis* in *H. armigera* increases the efficacy of *M. anisopliae*. Similarly, the combined application of both biocontrol agents showed a significant effect on the following developmental stages of both instar larvae with no or little pupation and adult emergence.

In the current study, mycosis and sporulation on cadavers was higher at the low concentrations of conidia. However, mycosis and sporulation on cadavers depend on the method of exposure, the

conidial concentration and temperature. At high concentrations, numerous larvae died quickly, and the fungal sporulation was seen only in a few cadavers exhibiting small numbers of conidial production. A self-inhibiting mechanism at high concentrations of conidia was observed, which was also demonstrated by Tefera and Pringle[26] in their experiments. The same mechanism has been also reported by Garraway and Evans[46] for other species of fungi against various insect pests.

Our study suggests that *M. anisopliae* and *B. thuringiensis* can be used as potential biocontrol agents, especially when applied synergistically, for the management of the tomato leafminer. Moreover, pest activity will require intensive scouting to determine the correct application timings of control agents. Different *T. absoluta* instar larvae responded differently to the applied biocontrol agents, exhibiting various behavioral and physiological responses; more research is required to gain insight into the diversity of responses caused by these agents. However, the mortality obtained in the current laboratory bioassays may not predict the correct field mortality. Therefore, extensive field research is important to be conducted in order to examine the combined efficacy of *M. anisopliae* and *B. thuringienis* to develop and corroborate successful integrated pest management solutions against *T. absoluta*.

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Conflict of Interest
Authors declare no conflict of interest.

References

1. Cônsoli F.L., Parra J.R.P., Hassan S.A. Side-effects of insecticides used in tomato fields on the egg parasitoid Trichogramma pretiosum Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *J. Appl. Entomol*. 1998;122(1-5):43-47. doi:10.1111/j.1439-0418.1998.tb01459.x
2. Collavino M.D., Giménez R.A. Efficacy of imidacloprid to control the tomato borer (*Tuta absoluta* Meyrick). *Idesia (Arica)*. 2008;26(1):65-72. doi:10.4067/S0718-34292008000100009
3. Mantzoukas S., Milonas P., Kontodimas D., Angelopoulos K. Interaction between the entomopathogenic bacterium *Bacillus thuringiensis* subsp. *kurstaki* and two entomopathogenic fungi in bio-control of Sesamia nonagrioides (Lefebvre) (Lepidoptera: Noctuidae). *Ann. Microbiol*. 2013;63 (3):1083-1091. doi:10.1007/s13213-012-0565-x
4. Amer R.A.M., Hatem A.E., El-Sanady M.A. Microbial control of the tomato leaf-miner, *Tuta absoluta* (Povolny) (Lepidoptera: Gelechiidae) by biopesticides in relation to some biological parameters. *Egypt. J. Biol. Pest. Control*. 2012;22(2):161-167. https://www.cabi.org/ISC/abstract/20133080111.
5. Cagnotti C.L., Viscarret M.M., Riquelme M.B., Botto E.N., Carabajal L.Z., Segura D.F., Lopez S.N. Effects of X-rays on Tuta absoluta for use in inherited sterility programmes. *J Pest Sci* (2004). 2012;85(4):413-421. doi:10.1007/s10340-012-0455-9
6. Caparros Megido R., Haubruge E., Verheggen F.J. First evidence of deuterotokous parthenogenesis in the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *J. Pest. Sci*. (2004). 2012;85(4):409-412. doi:10.1007/s10340-012-0458-6
7. Cocco A., Deliperi S., Delrio G. Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. *J. Appl. Entomol*. 2013;137(1-2):16-28. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1439-0418.2012.01735.x.
8. Zappalà L., Biondi A., Alma A., Al-Jboory I.J., Arnò J., Bayram J., Chailleux, El-Arnaouty A., Gerling D., Guenaoui Y., Shaltiel-Harpaz L., Siscaro G., Stavrinides M., Tavella L., Aznar R.V., Urbaneja A., Desneux N. Natural enemies of the South American moth, Tuta

absoluta, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *J Pest Sci* (2004). 2013;86(4):635-647. https://link.springer.com/article/10.1007/s10340-013-0531-9.

9. Schnepf E., Crickmore N., Van Rie J., Lereclus D., Baum J., Feitelson J., Zeigler D.R., Dean D.H. Bacillus thuringiensis and its pesticidal crystal proteins. *Microbiol Mol Biol Rev.* 1998;62(3):775-806. http://www.ncbi.nlm.nih.gov/pubmed/9729609.
10. Zimmermann G. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and Beauveria brongniartii. *Biocontrol. Sci. Technol.* 2007;17(6):553-596. doi:10.1080/09583150701309006
11. Carner G.R., Yearian W.C. Development and use of microbial agents for control of Heliothis spp. in the USA. In: King EG, Jackson RD, eds. Proceedings of the Workshop on Biological Control of Heliothis: Increasing the Effectiveness of Natural Enemies, New Delhi, India, 11-15 November 1985. New Delhi, India: *Office of International Cooperation & Development, USDA.* 1989:467-481. http://agris.fao.org/agris-search/search.do?recordID=US201302706721.
12. Shan L.T., Feng M.G. Evaluation of the biocontrol potential of various Metarhizium isolates against green peach aphid Myzus persicae (Homoptera: Aphididae). *Pest Manag. Sci.* 2010;66(6):669-675. doi:10.1002/ps.1928
13. Scholte E.J., Njiru B.N., Smallegange R.C., Takken W., Knols G.J. Infection of malaria (Anopheles gambiae) and filariasis (Culex quinquefasciatus) vectors with the entomopathogenic fungus *Metarhizium anisopliae*. *Malar. J.* 2003;2:29-36.
14. Kumar P.A. Insect Pest Resistant Transgenic Crops. In: Advances in Microbial Control of Insect Pests. *Boston, MA: Springer US.* 2002:71-82. doi:10.1007/978-1-4757-4437-8_4
15. Bravo A., Soberón M., Gill S.S. *Bacillus thuringiensis*: Mechanisms and Use. In: Gilbert L.I., Gill S.S., eds. Insect Control Biological and Synthetic Agents. *Elsevier*; 2005:247-277. doi:10.1016/B0-44-451924-6/00081-8
16. Hilder V.A., Boulter D. Genetic engineering of crop plants for insect resistance-a critical review. *Crop Prot*. 1999;18(3):177-191. doi:10.1016/S0261-2194(99)00028-9
17. Bravo A., Gill S.S., Soberón M. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon*. 2007;49(4):423-435. doi:10.1016/J.TOXICON.2006.11.022
18. Frankenhuyzen K. van. Insecticidal activity of *Bacillus thuringiensis* crystal proteins. *J. Invertebr. Pathol.* 2009;101(1):1-16. doi:10.1016/j.jip.2009.02.009
19. Kumar P.A., Sharma R.P., Malik V.S. The Insecticidal Proteins of *Bacillus thuringiensis*. *Adv. Appl. Microbiol*. 1996;42:1-43. doi:10.1016/S0065-2164(08)70371-X
20. Bates S.L., Zhao J.Z., Roush R.T. Shelton A.M. Insect resistance management in GM crops: past, present and future. *Nat. Biotechnol*. 2005;23(1):57-62. doi:10.1038/nbt1056
21. Gao Y., Oppert B., Lord J.C., Liu C., Lei Z. *Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of Crioceris quatuordecimpunctata to *Beauveria bassiana* infection. *J. Invertebr. Pathol.* 2012;109(2):260-263. doi:10.1016/j.jip.2011.12.003
22. Wakil W., Ghazanfar M.U., Riasat T., Qayyum M.A., Ahmed S., Yasin M. Effects of interactions among *Metarhizium anisopliae*, *Bacillus thuringiensis* and chlorantraniliprole on the mortality and pupation of six geographically distinct *Helicoverpa armigera* field populations. *Phytoparasitica*. 2013;41(2):221-234. doi:10.1007/s12600-012-0282-9
23. Kryukov V.Y., Khodyrev V.P., Yaroslavtseva O.N., Kamenova A.S., Duisembekov B.A., Glupov V.V. Synergistic action of entomopathogenic hyphomycetes and the bacteria *Bacillus thuringiensis* ssp. morrisoni in the infection of Colorado potato beetle *Leptinotarsa decemlineata*. *Appl. Biochem. Microbiol*. 2009;45(5):511-516. doi:10.1134/S000368380905010X
24. Quesada-Moraga E., Navas-Cortés J.A., Maranhao E.A.A., Ortiz-Urquiza A., Santiago-Álvarez C. Factors affecting the occurrence and distribution of entomopathogenic

fungi in natural and cultivated soils. *Mycol. Res*. 2007;111(8):947-966. doi:10.1016/j.mycres.2007.06.006

25. Ma X.M., Liu X.X., Ning X., Zhang B., Han F., Guan X.M., Tan Y.F., Zhang Q.W. Effects of Bacillus thuringiensis toxin Cry1Ac and Beauveria bassiana on Asiatic corn borer (Lepidoptera: Crambidae). *J Invertebr Pathol.* 2008;99(2):123-128. doi:10.1016/j.jip.2008.06.01426. Tefera T., Pringle K.L. Food consumption by Chilo partellus (Lepidoptera: Pyralidae) larvae infected with *Beauveria bassiana* and *Metarhizium anisopliae* and effects of feeding natural versus artificial diets on mortality and mycosis. *J. Invertebr. Pathol.* 2003;84(3):220-225. doi:10.1016/j.jip.2003.11.001
27. Riasat T., Wakil W., Ashfaq M., Sahi S.T. Effect of *Beauveria bassiana* mixed with diatomaceous earth on mortality, mycosis and sporulation of Rhyzopertha dominica on stored wheat. *Phytoparasitica.* 2011;39(4):325-331. doi:10.1007/s12600-011-0164-6
28. Abbott W.S. A Method of Computing the Effectiveness of an Insecticide. *J. Econ. Entomol.* 1925;18(2):265-267. doi:10.1093/jee/18.2.265a
29. Vega-Aquino P., Sanchez-Peña S., Blanco C.A. Activity of oil-formulated conidia of the fungal entomopathogens Nomuraea rileyi and Isaria tenuipes against lepidopterous larvae. *J. Invertebr. Pathol*. 2010;103(3):145-149. doi:10.1016/j.jip.2009.12.002
30. Tefera T., Pringle K.L. Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* for Controlling Chilo partellus (Lepidoptera: Crambidae) in Maize. *Biocontrol. Sci. Technol.* 2004;14(8):849-853. doi:10.1080/0958315041000172707
31. Nguyen N.T.H., Borgemeister C., Poehling H.M., Zimmermann G. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. *Biocontrol. Sci. Technol.* 2007;17(8):853-864. doi:10.1080/09583150701546375
32. Sasidharan K.R., Varma R.V. Laboratory evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against Indarbela quadrinotata Walker (Lepidoptera: Metarbelidae)-a key pest of Casuarina equisetifolia L. in Tamil Nadu. *J. Biol. Control*. 2005;19(2):197-199. https://www.cabdirect.org/cabdirect/abstract/20063021156.
33. Inglis G.D., Goettel M.S., Butt T.M., Strasser H. Use of Hyphomycetous Fungi for Managing Insect Pests. In: Butt T.M., Jackson C., Magan N., eds. Fungi as Biocontrol Agents. Progress, Problems and Potential. *Wallingford, UK: CABI Publishing.* 2001:23–69. https://pdfs.semanticscholar.org/27e8/a02de5adf147f5f6df1c953bf907d4d6569a.pdf.
34. Wilson K., Cotter S.C., Reeson A.F., Pell J.K. Melanism and disease resistance in insects. *Ecol Lett*. 2001;4(6):637-649. doi:10.1046/j.1461-0248.2001.00279.x
35. Hafez M., Zaki F.N., Moursy A., Sabbour M. Biological effects of the entomopathogenic fungus, *Beauveria bassiana* on the potato tuber moth *Phthorimaea operculella* (Seller). *Anzeiger fur Schadlingskd Pflanzenschutz Umweltschutz*. 1997;70(8):158-159. doi:10.1007/BF01907353
36. Vandenberg J.D., Jackson M.A., Lacey L.A. Relative Efficacy of Blastospores and Aerial Conidia of Paecilomyces fumosoroseus against the Russian Wheat Aphid. *J. Invertebr. Pathol.* 1998;72(2):181-183. doi:10.1006/jipa.1998.4772
37. Herbert D.A., Harper J.D. Bioassay of a β-exotoxin of *Bacillus thuringiensis* against Heliothis zea larvae. *J. Invertebr. Pathol*. 1985;46(3):247-250. doi:10.1016/0022-2011(85)90065-5
38. Zehnder G.W., Gelernter W.D. Activity of the M-ONE Formulation of a New Strain of *Bacillus thuringiensis* Against the Colorado Potato Beetle (Coleoptera: Chrysomelidae): Relationship Between Susceptibility and Insect Life Stage. *J. Econ. Entomol*. 1989;82(3):756-761. doi:10.1093/jee/82.3.756
39. Lacey L.A., Horton D.R., Chauvin R.L., Stocker J.M. Comparative efficacy of *Beauveria bassiana*, *Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. *Entomol. Exp. Appl*. 1999;93(2):189-200.

doi:10.1023/A:1003831112208

40. Ahmad S. Enzymatic Adaptations of Herbivorous Insects and Nites to Phytochemicals. *J. Chem. Ecol*. 1986;12(2):533-560. https://link.springer.com/content/pdf/10.1007/BF01020571.pdf.
41. Mullin C.A. Adaptive relationships of epoxide hydrolase in herbivorous arthropods. *J. Chem. Ecol.* 1988;14(10):1867-1888. doi:10.1007/BF01013483
42. Wraight S.P., Ramos M.E. Synergistic interaction between *Beauveria bassiana* and *Bacillus thuringiensis* tenebrionis-based biopesticides applied against field populations of Colorado potato beetle larvae. *J. Invertebr. Pathol.* 2005;90(3):139-150. doi:10.1016/j.jip.2005.09.005
43. Lewis L.C., Berry E.C., Obrycki J.J., Bing L.A. Aptness of insecticides (*Bacillus thuringiensis* and carbofuran) with endophytic *Beauveria bassiana*, in suppressing larval populations of the European corn borer. *Agric. Ecosyst. Environ.* 1996;57(1):27-34. doi:10.1016/0167-8809(95)01011-4
44. Furlong M.J., Groden E. Starvation induced stress and the susceptibility of the Colorado potato beetle, *Leptinotarsa decemlineata*, to infection by *Beauveria bassiana*. *J. Invertebr. Pathol*. 2003;83(2):127-138. doi:10.1016/S0022-2011(03)00066-1
45. Lawo N.C., Mahon R.J., Milner R.J., Sarmah B.K., Higgins T.J. V., Romeis J. Effectiveness of *Bacillus thuringiensis*-transgenic chickpeas and the entomopathogenic fungus *Metarhizium anisopliae* in controlling *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Appl. Environ. Microbiol*. 2008;74(14):4381-4389. doi:10.1128/AEM.00484-08
46. Garraway M.O., Evans R.C. Fungal Nutrition and Physiology. New York, USA: *John Wiley and Sons.* 1984. doi:0471058440